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# Reproductive Performance in Pre- or Pubertal Female Rats after the First Ovulation

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## Summary

The experiment was designed to survey the reproductive performance in female rats of pre-puberty with PMS-induced ovulation and of puberty with natural ovulation.

3 IU of PMS was injected to rats of 25 or 31 days of age ("PMS 25" or "PMS 31") followed by mating with fertile males. The mating and conception rate and the growth of pups were examined afterwards. Also pubertal or adult rats were examined concerning the similar items as the PMS-treated ones. The degree of ova development during early gestation was examined by uterine perfusion at 4, 7 and 10th day of gestation. Progesterone (4 mg) and estrone (1 $\mu$g) were injected into some of the "PMS 25" group after mating to examine if gestation could be maintained.

Performance in the 2nd reproductive cycle was also investigated on "PMS 25" or "PMS 31".

Results were as follows:

1) The mating and conception rate in "PMS 31" (100%, 91%) and pubertal rats (100%, 73%) was normal as in adult rats (100%, 100%) but was low in "PMS 25" (44%, 29%). In "PMS 25" the development of fertilized ova was delayed during the implantation stage, as indicated by recovery of ova on the 7th day of gestation.

By injecting progesterone (4 mg) and estrone (1 $\mu$g) to "PMS 25" from the 4th to 13th day of gestation, the pregnancy was completed in all rats. In the 2nd reproductive cycle, all groups functioned normally indicating that the gestation induced in prepubertal rats with PMS treatment did not affect the succesive reproduction.

It has been well known since earlier observation (1, 2) that ovulation occurs steadily in prepubertal rats when they are singly injected with PMS (pregnant mare serum gonadotropin). Nuti and Meyer (3) recently reported that PMS-treated immature rats could conceive, maintain fetuses to term and deliver live pups in a normal manner and could nurse pups.

Umezu (4) observed that the first estrus in smear after vaginal opening was

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accompanied with ovulation in pubertal rats.

Uematsu *et al.* (5) showed that female rats in which puberty occurred naturally had the ability of reproductive performance as well as adult ones.

These reports show that the pre- or pubertal rats have a full ability of reproduction at the time of the first ovulation whether it is natural or induced.

This experiment was designed to ascertain whether or not female rats of pubertal and prepubertal stage have the potency of the successive reproduction and also, to know from what day of the prepubertal age rats begin to have the reproductive ability.

## Materials and Methods

Female rats of the Wistar strain bred in our laboratory were used in this experiment. All animals were weaned at 21 days of age and kept in an air-conditioned room with controlled illumination of 12 hr. day and 12 hr. night. These rats were provided food (Piggold: Zenno) and water *ad libitum*.

1) Reproductive performance of PMS-primed or pubertal rats.

i) PMS-primed rats.

Three IU/0.1 ml saline of PMS (P-mex: Sankyo Zoki) were injected s.c. to rats of 25 or 31 days of age, which were called the "PMS 25" or "PMS 31" groups.

On the second afternoon after injection, the vagina of each immature rat was opend by gently pushing with a cotton stick and co-existed with 2 fertile males.

On the next morning, mating was confirmed by the presence of plug or sperm in vagina. That day was defined as the 1st day of pregnancy.

The mating rate, conception rate, gestation period, and the number and body weight of pups at term or at weaning were recorded for these rats. The number of pups per mother at term was adjusted to 7.

Virgin cycling rats were also mated to compare the data on the reproductive performance.

ii) Pubertal rats.

Immature female rats were co-existed with the ratio of a female to 2 males after 30 days of age and the vaginal opening was checked every morning. After the vaginal opening, smear was taken to confirm mating by the existence of sperm or plug. These rats were put into the "Puberty" group. The observation on the pregnancy and the parturition in this group were made as in i).

2) Development of fertilized ova in PMS-primed rats.

As a means to examine the cause of low conception rate, as mentioned later, rats of "PMS 25" group were killed between 1 and 4 P.M. on the 4, 7 and 10th days of gestation. The weight of ovaries and uterus were estimated with a torsion balance and the number of implantation sites was counted. The degree of the development of fertilized ova was examined under the light microscope after uterine

perfusion. The same observation was made also in adult rats.

3) Effect of sex steroids on the maintenance of gestation in PMS-primed rats.

Rats of the "PMS 25" group when mating was confirmed were injected s.c. progesterone (4 mg/0.2 ml sesame oil: Nakarai Kagaku) together with estrone (1 $\mu$g/0.2 ml s.o.: Tokyo Kasei) once a day from 4th to 13th day of gestation. The dose of the hormones followed the report of Takeuchi *et al.* (6). These rats were observed on the conception rate, gestation period, and the number and body weight of pups at term.

4) Performance of PMS-primed rats in the second reproductive cycle.

After parturition in the first reproductive cycle, rats of "PMS 25" or "PMS 31" groups were remated at the first proestrus after weaning pups (3 weeks after parturition).

Rats which delivered pups but could not nurse them were also remated around 3 weeks after parturition.

The performance of the rats in the 2nd reproductive cycle was observed concerning mating and conception and the number and body weight of pups at term or at weaning.

Adult rats were also remated to compare the data on the 2nd reproductive cycle.

Statistical analysis of results was carried out with Student's t-test or $X^2$ test.

## Results

1) Reproductive performance of PMS-primed or pubertal rats.

Results were shown in Table 1. The body weight of female rats at mating in the 4 groups was heavier with advance of age ($P<0.01$). The mating rate of "PMS 25" (43.8%) was significantly, lower than that of the remaining groups (100%) ($P<0.01$). In "Puberty", the days of the vaginal opening, the first estrus in smear and the first mating were 35.9±0.5, 38.8±0.8 and 39.2±0.8 (means± S.E.), respectively.

In 15 out of 22 rats, mating was recognzied at the first estrus smear. The estrus smear continued for 2 days in 6 rats and for 3 days in 1 rat. Mating in these animals was recognzied on the last day of the estrus smear.

The conception rate was signfiicantly low (28.6%) in "PMS 25", compared with that of the other groups ($P<0.01$).

The gestation period in these rats which came to term was not different among the 4 groups.

On the body weight of mother at term, "PMS 25" did not significantly differ with "PMS 31", indicating a greater growth rate of the former during pregnancy.

The number of pups was larger as the age of the mother increased ("PMS 25" vs "Puberty" $P<0.01$. "PMS 31" vs "Puberty" $P<0.05$. "PMS 25" vs "Adult"

TABLE 1. *Reproductive record of PMS-primed or pubertal female rats*

| | Group | | | |
|---|---|---|---|---|
| | PMS 25 | PMS 31 | Puberty | Adult |
| Body wt. at mating (g) | 73.5±2.3* | 89.4±1.2 | 111.2±4.0 | 237.9±7.0 |
| Mating rate | 14/32 | 11/11 | 22/22 | 13/13 |
| Conception rate | 4/14 | 10/11 | 16/22 | 13/13 |
| Gestation period | 22.0±0.0 | 22.8±0.1 | 21.6±0.3 | 22.0±0.7 |
| Body wt. of mother at parturition (g) | 161.5±5.3 | 160.7±3.1 | 173.7±6.1 | 263.5±8.2 |
| Number of pups per mother at term | 6.8±0.3 | 8.1±0.4 | 9.6±0.4 | 10.1±0.6 |
| Body wt. of pup at birth (g) | 6.2±0.2 | 5.2±0.1 | 5.4±0.2 | 5.7±0.2 |
| Body wt. of mother at weaning (g) | 188.0±7.4 | 195.3±3.5 | | 202.3±6.9 |
| Nursing rate | 4/4 | 6/10 | | 9/13 |
| Number of pups per mother at weaning | 6.5±0.6 | 6.0±0.1 | | 4.6±0.6 |
| Body wt. of pups at weaning | 38.6±4.0 | 30.7±2.4 | | 35.1±4.2 |

Three IU PMS were injected s.c. into rats of 25 and 31 days of age, which were called "PMS 25" or "PM 31". On the second afternoon after the injection, rats were co-existed with males. Immature rats were co-existed with males after 30 days of age and checked vaginal opening and mating. The rats were surveyed concerning the items represented in the Table.

* Mean±standard error

or "PMS 31" vs "Adult" $P<0.01$).

The body weight of pups at term of "PMS 25" are heavier than that of "PMS 31" ($P<0.01$), but no significant difference was shown in any other assortment among the 4 groups. The nursing rate of "PMS 25", "PMS 31" and "Adult" was 100, 60 and 69%, respectively, indicating a rational value.

2) Development of fertilized ova in PMS-primed rats.

Results were shown in Table 2. The implantation site was not obvious in either group on the 4th day of gestation. Ova were recovered by uterine perfusion in 2 out of 2 PMS-primed rats and in 1 out of 2 adults. In "PMS 25", 2 ova and 1 ovum were in the stage of molura and 4 cell, respectively in a) rat, whereas all 3 ova were degenerated in b) rat. In "Adult", all ova in a) were in the stage of late blastocyst which lacked the zona pellucida.

At the 7th day of gestation, the implantation sites were clearly recognized in both groups. Ova were recovered in all 3 rats of "PMS 25" but not in adults by uterine perfusion. All the recovered ova were in the stage of the late blastocyst in c) and e) rat. One ovum was a normal late blastocyst and the remaining 4 were degenerated in d) rat.

At the 10th day of gestation, each of implantation sites was remarkably small in h) rat of "PMS 25" and suggested to be on the way to degeneration. The sites were clearly recognized as in "Adult" but were still smaller in the remaining f) and g) rats.

The embryonic loss which was estimated by the balance of the number of corpus

TABLE 2. *Development of fertilized ova in PMS-primed rats*

| | Day of pregnancy | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | | | | 7 | | | | | | 10 | | | | | | | |
| | PMS 25 | | Adult | | PMS 25 | | | Adult | | | PMS 25 | | | Adult | | | | |
| Sign of rat | a | b | a | b | c | d | e | c | d | e | f | g | h | f | g | | | |
| Body wt. (g) | 71 | 66 | 225 | 180 | 90 | — | 95 | 195 | 210 | 250 | 124 | 110 | 110 | 227 | 210 | | | |
| Number of recovered ova | 3 | 3 | 3 | 0 | 3 | 5 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| Stage of ova | 2. mo 1.4 cell | 3 deg | 3.1.b | | 3.1.b. | 1.1.b. 4 deg | 5.1.b. | | | | | | | | | | | |
| Number of implantation site | n.d. | n.d. | n.d. | n.d. | 6 | 8 | 7 | 10 | 10 | 13 | 8 | 8 | 6 | 14 | 10 | | | |
| Number of C.L. | 9 | 9 | 12 | 12 | 7 | 9 | 8 | 10 | 10 | 13 | 10 | 8 | 7 | 14 | 13 | | | |
| Ovarian wt. (mg) | 21 | 16 | 36 | 32 | 16 | 28 | 22 | 47 | 53 | 52 | 42 | 32 | 36 | 58 | 76 | | | |
| Uterine wt. (mg) | 95 | 84 | 347 | 325 | 140 | 72 | 96 | 374 | 490 | 467 | 594 | 698 | 150 | 1300 | 1114 | | | |

Rats with PMS-priming at 25 days of age were killed at 1 to 4 P.M. on the 4, 7 or 10th day of gestation. Adult rats were also killed on the same schedule as "PMS 25". The stage of ova was examined after uterine perfusion. mo: ova in the stage of morulla. 4 cell: ova in the stage of 4 cell. l.b.: ova in the stage of late blastocyste. deg: degenerated ova.

luteum and implantation sites was observed to be 3/3 of "PMS" and 0/3 of "Adult" at the 7th day of gestation, and to be 2/3 of "PMS" and 1/2 of "Adult" at the 10th day.

The weight of ovaries and uterus in "PMS 25" was lighter than "Adult" at every stage in early gestation.

3) Effect of sex steroids on the maintenance of gestation in PMS-primed rats.

The results were shown in Table 3. All rats of "PMS 25" treated with the sex steroids proceeded to normal gestation, whereas only 28.6% in the group without steroids did.

TABLE 3. *Effect of sex steroids on conception rate in PMS-primed rats*

| | No. of rats | Body wt. at mating (g) | Conception rate | Gestation period (day) | Number of pups at term | Body wt. of pups at term (g) |
|---|---|---|---|---|---|---|
| Steroids-treated | 4 | 73.3±2.3* | 4/4 | 22.3±0.3 | 6.3±1.0 | 6.1±0.1 |
| Non-treated | 14 | 73.5±8.4 | 4/14 | 22.0±0.0 | 6.8±0.3 | 6.2±0.2 |

Rats which had had PMS-priming at 25 days and for which mating had been confirmed were injected with s.c. progesterone (4 mg/0.2 ml sesame oil) together with estrone (1 μg/0.2 ml) once a day from 4th to 13th day of gestation in "Steroid-treated".

Results were recorded on the items represented in the table.

* Mean±standard error

The number and body weight of pups at term and the gestation period were not different between the both groups.

4) Performance of PMS-primed rats in the second reproductive cycle.

The results were shown in Table 4. The body weight of rats in the PMS groups at the 2nd mating was beyond 190 gr., which is normal weight for the first copulation in our laboratory. The mating and conception rates of "PMS 25" in the 2nd reproductive cycle were obviously superior to those in the 1st one.

These records in the 2nd reproductive cycle in "PMS 31" or "Adult" did not differ with those in the 1st one.

The number of pups at term tended to be larger at the 2nd reproductive cycle compared with the 1st cycle in every group and the number was significantly different in "PMS 25" and "PMS 31" ($P<0.01$).

The nursing capacity of mothers in the 2nd reproductive cycle slightly improved in "PMS 31" and "Adults".

The number of survival pups at weaning in the 2nd cycle also increased in "PMS 31" and "Adult", but did not in "PMS 25". The body weight of pups at weaning in the 2nd cycle tended to be heavier in evey group than in the 1st one, indicated by the significant difference in "PMS 31" ($P<0.05$).

TABLE 4. *Reproductive record at 2nd delivery in PMS-primed rat*

| | Groups | | |
|---|---|---|---|
| | PMS 25 | PMS 31 | Adult |
| Body wt. at mating (g) | 195.5±4.9* | 201.9±4.5 | 263.1±7.6 |
| Rate of mating | 4/4 | 9/10 | 11/13 |
| Rate of conception | 4/4 | 7/9 | 8/11 |
| Body wt. of mother at parturition | 240.0±5.4 | 233.9±5.0 | 297.5±9.1 |
| Number of pups per mother at term | 11.3±0.7 | 11.6±0.5 | 11.2+0.7 |
| Body wt. of pup at birth | 5.8±0.2 | 4.9±0.2 | 5.7±0.2 |
| Body wt. of mother at weaning | 243.3±2.8 | 238.7±5.0 | 287.6±5.8 |
| Rate of nursing | 4/4 | 5/7 | 10/11 |
| Number of pups per mother at weaning | 6.0±0.8 | 7.8±0.2 | 6.5±0.2 |
| Body wt. of pups at weaning | 45.6±2.8 | 41.1±2.6 | 40.1±3.0 |

Rats which had succeeded in parturition was remated 3 weeks after parturition and the 2nd reproductive performance in the 2nd reproductive cycle was observed on the items represented in the table.

* Mean±standard error

## Discussion

In rats of "PMS 31" and "Puberty", the mating and conception rate, the gestation period and the weight of delivered pups were almost the same as with mature rats.

The records on the reproductive performance in "PMS 31" almost consist with the report of Nuti & Meyer in which 8 IU PMS was injected to rats of 30 days of age (3).

Also the records of "Puberty" almost agree with those of Uematsu *et al.* (5). The discharge of the ovulating hormone from the pituitary occurs consistently on the proper time in the afternoon of the proestrus day in both pubertal (7～9) and PMS-treated rat (10, 11) and ovulation takes place early morning at the estrus (12, 13).

Rats usually mate at the time between gonadotropin discharge and ovulation.

The results in the present experiment show that female rats have the ability to reproduce at the first ovulation if the animals are at least 30 days of age. The mating or conception rate was low in "PMS 25". We often observed that adult male rats ejaculated extravaginally, as females were too small to be fixed to insert the penis into the vagina.

This seems to be the main reason for the low mating rate in "PMS 25". Although the conception rate was very low in that group, rats which proceeded gestation to parturition tended to be similar in gestation period and in body weight of pups, and to be slightly superior in nursing ability. This indicates that the successive reproductive performance is normal even in "PMS 25" except for the low mating or conception rate.

The low conception rate in "PMS 25" may be caused by the insufficiency of the implantation and the early embryonic development.

This idea is derived from the fact that fertilized ova were recovered in "PMS 25" through uterine perfusion at the 7th day of gestation when ova were never obtained in "Adult" and that the development of recovered ova was delayed to the 4th day in "PMS 25".

All of "PMS 25" could complete normal pregnancy to parturition if a combination of progesterone and estrone were injected from 4th to 13th day of gestation. This way shows that the embryo of the earlier stage can normally develop even in rats of "PMS 25" when the appropriate dose of sex steroids was supplied for the appropiate period. In adult ovariectomized rats, the normal implantation and embryonic development can be attained with exogenous sex steroids (6). This shows that the normal secretion of ovarian hormone is necessary for implantation and development of fertilized ova. From the result, it is suggested that the insufficiency of conception in "PMS 25" is not merely due to physical maldevelopment of uterus because of immaturity, but due to the insufficiency of hormone secretion by the ovary during early gestation after PMS treatment and mating procedure.

In the pregnancy of PMS-primed rat after 30 days of age the pattern of hormones seems to develop like that of adults (3).

In this experiment, however, it was not determined whether or not the ovary in "PMS 25" could secrete sufficient hormones to keep gestation. The appropriate combination and injected time of the sex steroids also remain to be investigated in detail. Rats which had come to parturition in the first

reproductive cycle had normal performance in the 2nd reproductive cycle, in which a rather slight improvement was observed on the mating and conception rate, the number and growth of pups and nursing rate.

Thus, it is confirmed that the gestation which was induced in immature rats with PMS treatment did not affect the next reproduction.

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